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[Continued on next page]

(54) Title: HYBRID AND TANDEM EXPRESSION OF NEISSERIAL PROTEINS

ΔG287-919-His
ΔG287-Orf46.1-His
ΔG287-953-His
ΔG287-961-His
ΔG287-230-His
ΔG287-936-His
ΔG287-287-His
ΔG287-287_{nz}-His
ΔG287- 741_{MC58} -His
ΔG287-741_{ET37}-His

ΔG287_{nz}-919-His
ΔG287_{nz}-953-His
ΔG287_{nz}-961-His
ΔG287_{nz}-287-His
ΔG287_{nz}-287_{nz}-His
ΔG287_{nz} - 741_{MC58} -His

ΔG287-919-Orf46.1-His
ΔG287-Orf46.1-919-His
919-287-Orf46-His
Orf46.1-287-919-His

961c-741_{MC58}-His
961c-983-His
961c-Orf46.1-His
961cL-741_{MC58}
961cL-287
961c-230-His
961c-936-His

ΔG741_{MC58}-961c-His
ΔG741_{MC58}-961-His
ΔG741_{MC58}-983-His
ΔG741_{MC58}-Orf46.1-His
ΔG741_{MC58}-741_{MC58}-His
ΔG741_{MC58}-741_{ET37}-His

919-287
953-287
919-Orf46.1-His

Orf46.1-287-His
Orf46.1-919-His
Orf46.1-741_{MC58}-His
Orf46.1-961-His
Orf46.1-961c-His
Orf46.1-983-His
Orf46.1-936-His
Orf46.1-230-His

230-741_{MC58}-His
230-Orf46.1-His
230-961-His
230-961c-His
936-741_{MC58}-His
936-Orf46.1-His
936-961-His
936-741_{ET37}-His

ΔG983-741_{MC58}-His
ΔG983-961c-His
ΔG983-961-His
ΔG983-Orf46.1-His

(57) Abstract: Two or more Neisserial proteins are joined such that they are translated as a single polypeptide chain. Hybrid proteins are represented by the formula NH_2-A-[-X-L-]$_n$-B-COOH where X is an amino acid sequence, L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and *n* is an integer greater than I. Proteins where each of the *n* -X- moieties shares sequence identity to each other -X- moiety, the protein is a 'tandem protein'.

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6	2	$\Delta G287_{2996}$	$(Gly)_6$	$\Delta G287_{394/98}$	-
7	2	$\Delta G287_{2996}$	$(Gly)_6$	$\Delta G287_{2996}$	-
8	2	$\Delta G287_{394/98}$	$(Gly)_6$	$\Delta G287_{394/98}$	-
9	2	$\Delta G287_{394/98}$	$(Gly)_6$	$\Delta G287_{2996}$	-
10	2	$\Delta G741_{MC58}$	-	$741_{394/98}$	$(His)_6$
11	2	$\Delta G741_{MC58}$	-	$741_{90/18311}$	$(His)_6$
12	2	$\Delta G741_{MC58}$	-	741_{95N477}	$(His)_6$

Proteins #1 to #5 have all been expressed in soluble form in *E.coli*. Expression levels were between 0.24 and 0.50 mg protein per litre of culture. The tandem proteins were purified and mixed with aluminium phosphate as an adjuvant. Tandem proteins #2, #4 and #5 adsorbed readily to aluminium phosphate; adsorption was less complete for tandem proteins #1 and #3.

Allelic variants – 741

Twenty-two polymorphic sequences of 741 were found (SEQ IDs 1 to 22). These and the MC58 sequence are aligned in Figure 1.

Allelic variants – NMB1343

Using PCR on 42 strains of meningococcus of various serogroups, the gene encoding NMB1343 protein was found in 24/42 and was absent in 18/42 strains (Table 1). The NMB1343 gene was sequenced for 10 of the NMB1343$^+$ strains (Table 1, column 3). The nucleic acid sequence (and thus amino acid sequence SEQ ID 23; GenBank AAF41718) was identical in all 10 strains.

NMB1343 was also detected in two strains of *N.gonorrhoeae* (F62 and SN4). The amino acid sequence from gonococcus is SEQ ID 24. An alignment with the meningococcal sequence is:

```
                 . . . .10 . . . .20 . . . .30 . . . .40 . . . .50
Ng     1:INNLWEISYLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKAT: 50
Nm     1:~~~~~MGNFLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKAT: 45

                 . . . .60 . . . .70 . . . .80 . . . .90 . . . 100
Ng    51:HGPSVKNAVYAHQIETDLYDGCYISTTTDKEIAKKFATSSGIENGYIYVL:100
Nm    46:HGPSVKNAVYAHQIETGLYDGCYISTTTDKEIAKKFATSSGIENGYIYVL: 95

                 . . . 110 . . . 120 . . . 130 . . . 140 . . . 150
Ng   101:NRDLFGQYSIFEYEVEHPENPDEKEVTIRAEDCGCIPEEVIIAKELIEIN:150
Nm    96:NRDLFGQYSIFEYEVEHPENPNEKEVTIRAEDCGCIPEEVIIAKELIEIN:145
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An alignment of the corresponding nucleotide sequences is shown in Figure 2. This shows that the gonococcal sequence has a 4mer insertion in the 5' region of the NMB1343 gene which causes a frameshift and consequent loss of the 5' methionine residue.

Domain deletion – 961

961 is not present in the *N.meningitidis* serogroup A genome sequence [81], even though the surrounding regions are conserved (>90%) between serogroups A and B. References 11 and 12 disclose polymorphic forms of 961. The gene was found to be present in 91% of serogroup B strains belonging to hypervirulent lineages ET-5, ET-37 and cluster A4, but was absent in all strains of lineage 3 tested. Most of the serogroup C strains tested were positive even if not belonging to hypervirulent lineages. The same was true for the serogroup B strains with serotype 2a and 2b. For serogroup A, one strain belonging to subgroup III was positive whereas the other two strains belonging to subgroup IV-1 were negative. 961 was absent in *N.gonorrhoeae* and in commensal species *N.lactamica* and *N.cinerea*.

Figures 4 and 5 show domains in protein 961.

When the anchor region (domain 9) of protein 961 is deleted ('961cL') and expressed in *E.coli*, the protein is exported in the periplasm and secreted in the supernatant of the culture.

To investigate this further, deletion mutants in the C-terminal region of 961 were constructed (961cL-Δaro, 961cLΔcc, 961aL, 961aL-Δ1, 961aL-Δ2, 961aL-Δ3) on the basis of structural features (deletions of aromatic residues in the cases of 961cΔaro mutant, and of coiled-coil regions for the others). These were analysed for expression and secretion into the periplasm and the supernatant of the culture. In all of these deletion mutants, the protein is produced in large amount, is present in periplasmic fraction, and is released in the supernatant of the culture.

ΔG287 – cross-strain bactericidal activity

287 was cloned for five different *N.meningitidis* serogroup B strains and was manipulated to delete the N-terminus up to the end of the poly-glycine region and to introduce a C-terminal his-tag. This gave five ΔG287 proteins. These were adjuvanted with FCA and used to raise immune sera in mice, which were then tested for bactericidal activity against all five serogroup B strains and also against serogroup A and C strains. Bactericidal titres were as follows:

Protein strain	Sera tested for bactericidal activity against strain *						
	2996	**BZ232**	**MC58**	**1000**	**394/98**	**F6124**	**BZ133**
2996	**16000**	128	4096	4096	1024	8000	16000
BZ232	>8000	**256**	2048	8000	2048	16000	8000
MC58	>8000	64	**>8000**	8000	2048	8000	8000
1000	>8000	64	4096	**8000**	1024	16000	16000
394/98	>16000	128	16000	>2048	**>16000**	-	-

* titres against homologous strain shown in bold

Refolding

To improve the levels of soluble protein for some hybrid proteins, alternative refolding protocols to those disclosed in reference 2 were adopted.

Inclusion bodies (IBs) were isolated as follows:

1. Homogenize cells (5g wet weight) in 25 ml 0.1 M Tris-HCl pH 7, 1mM EDTA, at 4°C using an ultraturrax (10 000 rpm)
2. Add 1.5mg lysozyme per gram cells, mix shortly with an ultraturrax, and incubate at 4°C for 30 min.
3. Use sonication or high-pressure homogenization (French press) to disrupt the cells.
4. To digest DNA, add $MgCl_2$ to a final concentration of 3mM and DNase to a final concentration of 10μg/ml, and incubate for 30 min at 25°C
5. Add 0.5 vol. 60 mM EDTA, 6% Triton X-100, 1,5M NaCl pH7, to the solution, and incubate for 30 min at 4°C.
6. Spin down inclusion bodies by centrifugation at 31000*g* (20 000 rpm) for 10 min, 4°C.
7. Resuspend pellet in 40 ml 0.1 M tris-HCl pH 7, 20mM EDTA, using an ultraturrax
8. Repeat centrifugation step 6.
9. The inclusion body pellet may be used, or stored frozen at –20°C.

Hybrid proteins were expressed in *E.coli* as follows:

Protein	Culture volume (litres)	Flask volume (litres)	Temp (°C)	Final OD_{600}	Inclusion body yield (w/w)
ORF46.1-961-His	1	2	37	1.51	33.2%
ORF46.1-961c-His	1	2	37	1.6	28.3%
961c-ORF46.1His	1	2	37	1.18	23.5%
orf46.1-741 His	5	5	37	12.42	35.2

The pellets were solubilised, refolded, ultrafiltered, dialysed, and protein was then purified:

ORF46.1-961-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 1 mg/ml. To refold the protein, 2 ml of solubilised protein was diluted in 400 ml of refolding buffer (0.1M Tris HCl,1M L-arginine, 2mM EDTA pH 8.2) and incubated for 1 hour at 15°C, resulting in a protein concentration of 5μg/ml. Subsequently, another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 10 μg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 130 ml final volume. The

ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24hours against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5 The supernatant isolated after centrifugation was used for His-tag purification.

orf 46.1-961c-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 1 mg/ml. To refold the protein, 2 ml of the solubilised protein was diluted in 400 ml refolding buffer (0.5M Tris HCl,1M L-arginine,2 mM EDTA pH 8.2) and incubated for 1 h at 15°C, resulting in a protein concentration of 5μg/ml. Subsequently another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 10μg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 150 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24h against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5. The supernatant isolated after centrifugation was used for His-tag purification.

961c-orf46.1-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 1 mg/ml. To refold the protein, 2 ml of the solubilised protein was diluted in 400 ml refolding buffer (0.1M Tris HCl,0.5 M L-arginine,2 mM EDTA pH 8.2) and incubated for 1 h at 15°C, resulting in a protein concentration of 5 μg/ml. Subsequently another 2 ml of the solubilized protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 10 μg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 150 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24h against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5. The supernatant isolated after centrifugation was used for His-tag purification.

orf46.1-741-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 10 mg/ml. To refold, 2 ml of the solubilised protein was diluted in 400 ml of the refolding buffer (0.5M Tris HCl,0.7 M L-arginine,2 mM EDTA pH 7.2) and incubated for 1 h at 15°C, resulting in a protein concentration of

50μg/ml. Subsequently another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 100μg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 120 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24h against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5 The supernatant isolated after centrifugation was used for His-tag purification.

Compared with proteins purified as described in ref. 2, bactericidal assay titres were as follows:

Protein	Reference 2		Refolded		
	CFA	Aluminium hydroxide	Aluminium hydroxide	MF59	Aluminium phosphate
ORF46.1-961-His	8192	8192	32768	-	-
ORF46.1-961c-His	8192	128	<64	8192	-
961c-ORF46.1His	32768	1024	16384	-	-
orf46.1-741 His	<4	16	<4	256	-

Similar procedures were used for ORF46.1 to purify the protein from IBs when expressed with no His-tag ('ORF46.1K'):

Protein	Culture volume (litres)	Flask volume (litres)	Temp (°C)	Final OD_{600}	Inclusion body yield (w/w)
orf46.1K	5	5	37	13.7	29.4

IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 10 mg/ml. To refold, 2 ml of the solubilised protein was diluted in 400 ml of the refolding buffer (0.5M Tris HCl,0.7 M L-arginine,2 mM EDTA pH 7.2) and incubated for 1 hours at 15°C, resulting in a protein concentration of 50μg/ml. Subsequently another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 100μg/ml. The material was ultrafiltered using a 300ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30kDa cut-off (YM30) resulting in 120 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 12h against 10 L of 50mM sodium phosphate, 2mM EDTA, pH 7,2 buffer. A second dialysis of 24h against 10 L of the same buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5. The supernatant isolated after centrifugation was used for cationic exchange chromatography. The purification was done on a AKTA explorer chromatography

system (Amersham-Pharmacia Biotech) using a 5 ml HiTrap SP sepharose HP column (Amersham-Pharmacia Biotech). The flow rate applied was of 1.5 ml per minute. The column was washed with 35 ml of 50mM sodium phosphate buffer pH 7.2. A linear gradient (0-1 M NaCl) was performed using a 50mM sodium phosphate buffer pH 7.2. The protein eluted in two peaks at 92 mM and 380mM NaCl. The fractions constituting each peak were pooled and respectively named pool 1 and pool 2.

Compared with proteins purified as described in ref. 2, bactericidal assay titres when adjuvanted with aluminium hydroxide were improved from <4 to 1024. The titre using aluminium phosphate adjuvant with the refolded protein was 2048. ELISA titres were as follows:

Protein	Aluminium adjuvant	Elisa (M7)	SBA (2996)
Orf46.1k (pool 1)	Hydroxide 3.3mg/ml	1212	512
	Phosphate 0.6 mg/ml	154	1024
Orf46.1k (pool 2)	Hydroxide 3.3mg/ml	1085	1024
	Phosphate 0.6 mg/ml	250	1024

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

TABLE 1

Strain	1343	Sequence	Strain classification
72/00	+		ET5 B:15:P1.7,13,13a
30/00	+		ET5 B:15:P1.7,16
39/99	+		ET5 C:15:P1.7,16
95330	+		ET5 B:4:P1.15
M4102	+		ET5 nd
MC58(21)	+	+	ET5 B:15:P1.7,16b
BZ169(7)	+	+	ET5 B:NT:P1.16
BZ83(19)	+		ET5 B:15:-.-
CU385	+	+	ET5 B:4:P1.15
220173I	+		ET5 NG:4:P1.15
64/96	+	+	ET5 NG:15:P1.7,16 (carrier)
220173I	+		ET5 B:4:P1.15 (carrier)
ISS1071	+		nd B:15:P1.7,16 (ET5?)
BZ198(2)	+	+	lin.3 B:8:P1.1
980-2543	+	+	lin.3 B:NT:P1.4
16060	+	+	other B:4:P1.14 (carrier)
394-98	+		nd B:4:P1.4 (lin 3?)
ISS1106	+		nd B:4:P1.4 (lin.3?)
BZ133(10)	+	+	sub I B:NT:-.-
S3446	+	+	nd B:14:P1.23,14
ISS1001	+	+	nd B:14:P1.13
241175I	+		other NG:21:P1.16 (carrier)
171274I	+		other NG:15:- (carrier)
66/96	+		other B:17:P1.15 (carrier)
961-5945	-		A4
96217	-		A4
312294	-		A4
90/18311(24)	-		ET37
93/4286(25)	-		ET37
M986	-		ET37
1000(5)	-		other
NGE28(13)	-		other carrier
NGH38(14)	-		other carrier
BZ232(18)	-		other
F6124(23)	-		sub III A:-.-
C11	-		C:-
NMB	-		nd
8047	-		nd
ISS759	-		nd C:2b:P1.2
ISS1113	-		nd C:2:P1.5
65/96	-		nd 4:P1.14
2996(96)	-		nd B:2b:P1.5,2

CLAIMS

1. A hybrid protein having formula:

$$NH_2\text{-}A\text{-}[\text{-}X\text{-}L\text{-}]_n\text{-}B\text{-}COOH$$

wherein L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and *n* is an integer greater than 1, and X is either:

(a) an orf1, orf4, orf25, orf40, orf46.1, orf83, NMB1343, 230, 233, 287, 292, 594, 687, 736, 741, 907, 919, 936, 953, 961 or 983 amino acid sequence;

(b) an amino acid sequence having sequence identity to an amino acid sequence from (a); or

(c) an amino acid sequence comprising a fragment of an amino acid sequence from (a).

2. A hybrid protein having formula:

$$NH_2\text{-}A\text{-}[\text{-}X\text{-}L\text{-}]_n\text{-}B\text{-}COOH$$

wherein X is an amino acid sequence, L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, *n* is an integer greater than 1, and wherein a first X moiety ($-X_a-$) has one of the following amino acid sequences:

(d) the 446 even SEQ IDs (i.e. 2, 4, 6, ... , 890, 892) disclosed in reference 3.

(e) the 45 even SEQ IDs (i.e. 2, 4, 6, ... , 88, 90) disclosed in reference 4;

(f) the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241, disclosed in reference 5;

(g) the 2160 amino acid sequences NMB0001 to NMB2160 from reference 7; or

(h) an amino acid sequence disclosed in reference 1 or reference 2,

and a second -X- moiety ($-X_b-$), wherein $-X_b-$ has sequence identity to $-X_a-$ and/or $-X_b-$ comprises a fragment of $-X_a-$.

3. The hybrid protein of claim 1 or claim 2, wherein *n*=2.

4. The hybrid protein of claim 2, wherein $-X_a-$ is an orf46.1, 230, 287, 741, 919, 936, 953, 961 or 983 amino acid sequence.

5. The hybrid protein of claim 2, wherein $X_1, \ldots, X_n$ all have sequence identity to each other.

6. The hybrid protein of any preceding claim, wherein n=2, and wherein the -X- moieties are: ΔG287 and 230; ΔG287 and 936; ΔG287 and 741; 961c and 287; 961c and 230; 961c and 936;

961cL and 287; 961cL and 230; 961cL and 936; ORF46.1 and 936; ORF46.1 and 230; 230 and 961; 230 and 741; 936 and 961; 936 and 741; ΔG741 and 741; or ΔG287 and 287.

7. The hybrid protein of any preceding claim, wherein L has 20 or fewer amino acids.

8. The hybrid protein of any preceding claim, wherein L is a poly-glycine linker.

9. The hybrid protein of any preceding claim, wherein A has 40 or fewer amino acids.

10. The hybrid protein of any preceding claim, wherein B has 40 or fewer amino acids.

11. The hybrid protein of any preceding claim, wherein the -X- moieties have an amino acid sequence found in *N.meningitidis* serogroup B.

12. The hybrid protein of any preceding claim, wherein at least one -X- moiety is a 961 amino acid sequence in which one or more domains has been deleted.

13. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 1 to 38.

14. Nucleic acid encoding a protein of any preceding claim.

15. A composition comprising protein or nucleic acid according to any preceding claim.

16. A composition comprising two or more of the following proteins:
    (1) 287
    (2) 741
    (3) ORF46.1
    (4) 961
    (5) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=953
    (6) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=919
    (7) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=961
    (8) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=741
    (9) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=936, X_2=741

17. The composition of claim 16, comprising proteins (4), (5) and (9).

18. The composition of claim 17, wherein protein (4) comprises SEQ ID 31, protein (5) comprises SEQ ID 28 or SEQ ID 29, and protein (9) comprises SEQ ID 30.

19. The composition of any one of claims 15 to 18, further comprising:
    – a protein antigen from *N.meningitidis*;
    – an outer-membrane vesicle (OMV) preparation from *N.meningitidis*;
    – a saccharide antigen from *N.meningitidis*;
    – a saccharide antigen from *Streptococcus pneumoniae*;

- an antigen from hepatitis A, B or C virus;
- an antigen from *Bordetella pertussis*;
- a diphtheria antigen;
- a tetanus antigen;
- a protein antigen from *Helicobacter pylori*;
- a saccharide antigen from *Haemophilus influenzae*;
- an antigen from *N.gonorrhoeae*;
- an antigen from *Chlamydia pneumoniae*;
- an antigen from *Chlamydia trachomatis*;
- an antigen from *Porphyromonas gingivalis*;
- polio antigen(s);
- rabies antigen(s);
- measles, mumps and/or rubella antigens;
- influenza antigen(s);
- an antigen from *Moraxella catarrhalis*;
- an antigen from *Streptococcus agalactiae*;
- an antigen from *Streptococcus pyogenes*; and/or
- an antigen from *Staphylococcus aureus*.

20. The composition of any one of claims 15 to 19, further comprising a pharmaceutically acceptable carrier.

21. The composition of claim 20 for use as a medicament.

22. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of the composition of claim 20.

SEQUENCE LISTING

SEQ ID 1 – 741 from strain 1000

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTTPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 2 – 741 from strain 220173I (premature stop codon, though reliable sequence)

MTRSKPVNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQ
DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKIEHLK
SPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKA

SEQ ID 3 – 741 from strain 90/18311 (incomplete)

GLADALTAPLDHKDKSLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEV
DGQLITLESGEFQIYKQDHSAVVALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGK
AFSSDDPNGRLHYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLA
LFGDRAQEIAGSATVKIREKVHET

SEQ ID 4 – 741 from strain L93/4286 (incomplete)

VAADIGAGLADALTAPLDHKDKGLQSLMLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFD
FIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPDG
KAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGGEE
KGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 5 – 741 from strain 2996

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ

SEQ ID 6 – 741 from strain 30/00

KDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEF
QVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKL
TYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAG
SAEVKTVNGIRHIGLAAKQ

SEQ ID 7 – 741 from strain 312294

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ

SEQ ID 8 – 741 from strain 39/99 (incomplete)

DKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQ
VYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLT
YTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGS
AEVKTVNGIRHIGLAAKQ

SEQ ID 9 – 741 from strain 5/99

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 10 – 741 from strain 67/00

MTRSKPVNRTAFCCFSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQEQ
DPEHSGKMVAKRRFKIGDIAGEHTSFDKLPKDVMATYRGTAFGSDDAGGKLTYTIDFAAKQGHGKIEHLK
SPELNVELATAYIKPDEKHHAVISGSVLYNQDEKGSYSLGIFGGQAQEVAGSAEVETANGIHHIGLAAKQ

SEQ ID 11 – 741 from strain BZ169

LQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYK
QSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTI
DFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEV
KTVNGIRHIGLAAKQ

SEQ ID 12 – 741 from strain 72/00

LQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYK
QSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTI
DFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEV
KTVNGIRHIGLAAKQ

SEQ ID 13 – 741 from strain 93/114

MTRSKPVNRTAFCCFSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQEQ
DPEHSGKMVAKRRFKIGDIAGEHTSFDKLPKDVMATYRGTAFGSDDAGGKLTYTIDFAAKQGHGKIEHLK
SPELNVELATAYIKPDEKHHAVISGSVLYNQDEKGSYSLGIFGGQAQEVAGSAEVETANGIHHIGLAAKQ

SEQ ID 14 – 741 from strain 95N477

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 15 – 741 from strain 96217

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ

SEQ ID 16 – 741 from strain BZ133

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQVQ
DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDASGKLTYTIDFAAKQGHGKIEHLK
SPELNVDLAASDIKPDKKRHAVISGSVLYNQAEKGSYSLGIFGGQAQEVAGSAEVETANGIRHIGLAAKQ

SEQ ID 17 – 741 from strain BZ232

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 18 – 741 from strain C11

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 19 – 741 from strain M1090

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN

NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 20 – 741 from strain M1096

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTTPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 21 – 741 from strain M198/172

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQVQ
DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDASGKLTYTIDFAAKQGHGKIEHLK
SPELNVDLAASDIKPDKKRHAVISGSVLYNQAEKGSYSLGIFGGQAQEVAGSAEVETANGIRHIGLAAKQ

SEQ ID 22 – 741 from strain NGH38

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 23 – NMB1343 from ten meningococcal strains

MGNFLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKATHGPSVKNAVYAHQIETGLYDGCYIS
TTTDKEIAKKFATSSGIENGYIYVLNRDLFGQYSIFEYEVEHPENPNEKEVTIRAEDCGCIPEEVIIAKE
LIEIN

SEQ ID 24 – NMB1343 from gonococcus

INNLWEISYLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKATHGPSVKNAVYAHQIETDLYD
GCYISTTTDKEIAKKFATSSGIENGYIYVLNRDLFGQYSIFEYEVEHPENPDEKEVTIRAEDCGCIPEEV
IIAKELIEIN

SEQ ID 25 – NMB1343 nucleic acid sequence (gonococcus)

TCCGCCGCATTACCTTATAAAATAAAACATCCCTCTCAAGCAGTCTGATAATGTTTGGATTGCTTGAGAT
TGATGAGTGATGGTGTTAAATTCAAACTTTAAATTAATAACTTATGGGAAATTTCTTATTTATATAGAGG
CATTAGTTGCCAACAAGATGAGCAAAATAATGGACAGTTAAAACCTAAAGGTAATAAAGCTGAAGTTGCA
ATTCGTTATGATGGTAAGTTTAAATATGATGGTAAAGCTACACATGGTCCAAGTGTGAAGAATGCAGTTT
ACGCCCATCAAATTGAAACAGATCTATATGACGGATGTTATATATCTACGACAACAGACAAGGAAATTGC
CAAGAAATTTGCAACAAGCTCCGGCATCGAAAATGGCTATATATATGTTTTAAATAGAGATTTGTTTGGT
CAATATTCTATTTTTGAATATGAGGTTGAACATCCAGAAAACCCAGATGAGAAGGAAGTAACAATCAGAG
CTGAAGATTGTGGCTGTATTCCTGAAGAAGTGATTATTGCTAAAGAGTTGATAGAAATTAACTAAGTTGA
AAGGTCAATATAATGGCTTTAGTTGAATTGAAAGTGCCCGACATTGGCGGACACGAAAATGTAGATATTA
TCGC

SEQ ID 26 – NMB1343 nucleic acid sequence (meningococcus)

TCCGCCGCATTACCTTATAAAATAAAACATCCCTCTCAAGCAGTCTGATAATGTTTGGATTGCTTGAGAT
TGATGAGTAATGGTGTTAAATTCAACCTTTAAATTAATAACTTATGGGAAATTTCTTATATAGAGGCATT
AGTTGCCAACAAGATGAGCAAAATAATGGACAGTTAAAACCTAAAGGTAATAAAGCTGAAGTTGCAATTC
GTTATGATGGTAAGTTTAAATATGATGGTAAAGCTACACATGGTCCAAGTGTGAAGAATGCAGTTTACGC
CCATCAAATTGAAACAGGTCTATATGACGGATGTTATATATCTACGACAACAGACAAGGAAATTGCCAAG
AAATTTGCAACAAGTTCCGGCATCGAAAATGGCTATATATATGTTTTAAATAGGGATTTGTTTGGTCAAT
ATTCTATTTTTGAATATGAGGTTGAACATCCAGAAAACCCAAATGAGAAGGAAGTAACAATCAGAGCTGA
AGATTGTGGCTGTATTCCTGAAGAAGTGATTATTGCTAAAGAGTTGATAGAAATTAACTAAGTTGAAAGG
TCAATATAATGGCTTTAGTTGAATTGAAAGTGCCCGACATTGGCGGACACGAAAATGTAGATATTATCGC

SEQ ID 27 – linker

GSGGGG

SEQ ID 28 – preferred ΔG287-953 hybrid

MASPDVKSADTLSKPAAPVVAEKETEVKEDAPQAGSQGQGAPSTQGSQDMAAVSAENTGNGGAATTDKPK
NEDEGPQNDMPQNSAESANQTGNNQPADSSDSAPASNPAPANGGSNFGRVDLANGVLIDGPSQNITLTHC
KGDSCNGDNLLDEEAPSKSEFENLNESERIEKYKKDGKSDKFTNLVATAVQANGTNKYVIIYKDKSASSS
SARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALR
VQGEPAKGEMLAGTAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKAAI
DGNGFKGTWTENGGGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQDGSGGGGATYKVDEYH
ANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPVANLQSGSQHFTDHLKSADIFDAAQYPD
IRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLV
NVGMTKSVRIDIQIEAAKQ

SEQ ID 29 – ΔG287$_{NZ}$-953 hybrid

MASPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGGQDMAAVSEENTGNGGAAATDKPK
NEDEGAQNDMPQNAADTDSLTPNHTPASNMPAGNMENQAPDAGESEQPANQPDMANTADGMQGDDPSAGG
ENAGNTAAQGTNQAENNQTAGSQNPASSTNPSATNSGGDFGRTNVGNSVVIDGPSQNITLTHCKGDSCSG
NNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDGKNDKFVGLVADSVQMKGINQYIIFYKPKPTSFARFR
RSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEP
SKGEMLAGTAVYNGEVLHFHTENGRPSPSRGRFAAKVDFGSKSVDGIIDSGDGLHMGTQKFKAAIDGNGF
KGTWTENGGGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQDGSGGGGATYKVDEYHANARF
AIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPVANLQSGSQHFTDHLKSADIFDAAQYPDIRFVS
TKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMT
KSVRIDIQIEAAKQ

SEQ ID 30 – 936-ΔG741 hybrid

MKPKPHTVRTLIAAIFSLALSGCVSAVIGSAAVGAKSAVDRRTTGAQTDDNVMALRIETTARSYLRQNNQ
TKGYTPQISVVGYNRHLLLLGQVATEGEKQFVGQIARSEQAAEGVYNYITVASLPRTAGDIAGDTWNTSK
VRATLLGISPATQARVKIVTYGNVTYVMGILTPEEQAQITQKVSTTVGVQKVITLYQNYVQRGSGGGGVA
ADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFI
RQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGR
ATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEK
GSYSLGIFGGKAQEVAGSAEVKTVNGIRHIGLAAKQ

SEQ ID 31 – 961c

MATNDDDVKKAATVAIAAAYNNGQEINGFKAGETIYDIDEDGTITKKDATAADVEADDFKGLGLKKVVTN
LTKTVNENKQNVDAKVKAAESEIEKLTTKLADTDAALADTDAALDATTNALNKLGENITTFAEETKTNIV
KIDEKLEAVADTVDKHAEAFNDIADSLDETNTKADEAVKTANEAKQTAEETKQNVDAKVKAAETAAGKAE
AAAGTANTAADKAEAVAAKVTDIKADIATNKDNIAKKANSADVYTREESDSKFVRIDGLNATTEKLDTRL
ASAEKSIADHDTRLNGLDKTVSDLRKETRQGLAEQAALSGLFQPYNVG